

Technical Description of the Armored RNA SARS-CoV-2 E gene

For RUO (Research Use Only)

Caution: The vials containing the Armored RNA (ArRNA) should be stored at -20°C in the dark.

Stable at least 6 months in these conditions.

A vial contains 1.5×10^7 copies suitable for ~100 reactions

1. Rehydration of Lyophilized ArRNA

- Write the date on the vial before opening.
- Resuspend the ArRNA in 500µL RNase free water / vial.
- Homogenize by pipetting up and down in the glass vial a 50µL-volume 10 to 20 times
- Rehydrated ArRNA must be incubated at room temperature for 5 min before extraction
- The total preparation or only a fraction of the preparation can be used for extraction using the routine extraction protocol. The remaining volume can be stored at -20°C or -80°C.
- After extraction, the RNA can be used in the same conditions as for an *in vitro* transcript (used as positive control for the molecular assay, or aliquoted and stored at -80°C for optimal stability).

2. Protocol used for validation of the ArRNA at UVE (Unité des Virus Emergents, Marseille, France)

- 500 µL of RNase free water were added in a vial and the ArRNA was resuspended as described above.
- 400µL were used for Extraction on EZ1 (Qiagen) platform using EZ1 Virus Mini Kit v2.1 (cat 955134).
- Nucleic acids were eluted in 60µl, EA buffer is added to a final volume of 500µl, and 5µl was used for RT-PCR following the previously described protocol <https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf>